ORIGINAL ARTICLE

Plasma Renin in Women Using and Not Using Combined Oral Contraceptive

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Abstract

Background: Recent studies show that women on combined oral contraceptives (COC) present abnormal fasting lipid profile, increased postprandial lipemia, plasma C-reactive protein (CRP) and blood pressure (BP) compared to women not on combined oral contraceptives. Plasma renin is one of the factors responsible for abnormal BP.

Objectives: To assess plasma renin levels in women using or not using COC, the correlation between renin and CRP, as well as divergences in lipid profile.

Methods: A cross-sectional study with apparently healthy women aged 20 to 30, eutrophic, irregularly active, and with fasting triglycerides < 150 mg/dL. The sample was stratified into two groups: the No Combined Oral Contraceptive Group (NCOCG), comprised of women who did not use any type of hormone contraceptive, and the Combined Oral Contraceptive Group (COCG) comprised of women on low-dose COC for at least one year. After a 12-hour fast, 5 ml of blood was collected for renin dosing and PCR. Data were analyzed by the t-Test and bidirectional Mann-Whitney Test, both with significance < 0.05.

Results: We evaluated 44 women equally distributed between the groups, age 23 ± 1.2 years, BMI 21.0 ± 3.2 kg/m^2. Median and interquartile deviation of renin in the NCOCG and the COCG were, respectively, 0.5 (0.1-1.0) and 3.0 (2-6) ($p < 0.01$). A positive correlation between PCR and renin ($p < 0.01$ and $r = 0.68$) was found.

Conclusion: The plasma renin levels of women using COC were higher, with a strong correlation with CRP. (Int J Cardiovasc Sci. 2020; 33(3):208-214)

Keywords: Hypertension; Metabolism; Contraceptive, Agents; Risk Factors; Genetics; Dyslipidemias; Diabetes Mellitus; Sedentarism, Women's Health.

Introduction

Several risk factors for cardiovascular disease are shared by women: family history, smoking, dyslipidemia, obesity, diabetes mellitus, arterial hypertension, physical inactivity and, specifically, the use of combined oral contraceptives (COC).[1] Evidence indicates that, in this population, the use of low-dose COC adversely alters the fasting lipid profile,[2] increases postprandial lipemia[3] and increases plasma C-reactive protein levels.[4]

It is also believed that these lipid alterations cause changes in vascular reactivity raising blood pressure levels.[5,6] Researchers in the 1990s showed that women who used COC were more likely to develop high blood pressure compared to women who did not use COC.[7] In a prospective cohort study of approximately 70,000

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US nurses with a four-year follow-up (between 1989 and 1993), the relative risk of developing hypertension was 50% higher for current COC users compared to new users and 10% higher compared to long-term users.[8] Translating these into absolute numbers, this increase means 41 cases in every 10,000 women using COC/year. However, at that time, the COCs were not yet of low dose.

More recent studies, which did not aim to assess blood pressure (BP), did not find any statistical difference between the BP of women who used and did not use COC, although systolic and diastolic pressure was higher in women on COC. Despite these results, studies have not yet evaluated the subclinical response of hormonal changes that influence the blood pressure of women taking COC.

Some aspects such as endothelial dysfunction due to reduced nitric oxide intake and changes in the functioning of the renin-angiotensin aldosterone system (RAAS) influence the development of hypertension. RAAS is presented as an endocrine axis in which each component of a cascade is produced by different organs. An arrangement that provides an example of interaction of several organic systems, engaged to maintain hemodynamic stability.[9]

Therefore, the objective of the present study was to test the hypothesis that there is a difference between the plasma renin levels of women who use and do not use COC, as well as to determine their relationship with CRP.

Methods

The present study follows the same data collection protocol from previous studies conducted by our research group.[10] The study is characterized as a comparative cross-sectional observational study in which 44 irregularly active women aged 20 to 30, eutrophic, nulliparous, using COC for at least one year or not using COC were evaluated for at least six months to one year. COCs were considered low dose if they contained 15–30 microgram ethinyl estradiol associated with progestin.

To determine whether the volunteer was irregularly active, the International Short-Form Physical Activity Questionnaire developed by the WHO and the Centers for Disease Control and Prevention (CDC) was used. This questionnaire was used because it has the following advantages: it can be performed in two forms (short and long form), it allows estimating caloric expenditure, presents a more detailed classification, into sedentary, irregularly active, active and very active, besides making it possible to draw comparisons and adjustments to the Brazilian reality.[11]

Exclusion criteria were the presence of diabetes mellitus, dyslipidemia, hepatic dysfunction, glycemia above 99 mg/dL, systemic arterial hypertension, hypo or hyperthyroidism, renal diseases, polycystic ovary syndrome, use of anabolic or dietary supplements, hypo or hyperlipidic diet, history of alcoholism, smoking, use of lipid-lowering drugs, corticoids, diuretics or beta-blockers. For this study, those with total cholesterol > 220 mg/dL, low-density lipoprotein > 160 mg/dL or triglycerides > 150 mg/dL were considered dyslipidemic.

Ethical Criteria

Firstly, the research study was carried out in the Physiotherapy course of Faculdade Social da Bahia. All women who were willing to participate in the study were initially evaluated and those who agreed with the inclusion criteria and did not present any exclusion criteria were included in the study.

All of the research steps, such as the study objectives and the risks and benefits involved in the procedures, were explicitly detailed to the volunteers in a reader-friendly manner. The volunteers signed an informed consent form.

Throughout the study, the human research guidelines of the Declaration of Helsinki and Resolution 466/12 of the National Health Council were observed. This study was submitted and approved by the Research Ethics Committee with CAAE number 79549517.3.0000.5654.

Data Collection Protocol

Participants were divided into two groups. One group using COC (COCG) and one group without COC use (NCOCG). Initially, the volunteers answered a standard questionnaire and were submitted to physical examination. Both were conducted in order to collect general information about the sample characteristics. Physical examination included determination of resting BP, total body mass, height and waist circumference measurements.

To determine BP, the American Heart Association recommendations were followed, using a medium-sized tensiometer for the average adult, duly calibrated by the National Institute of Metrology (INMETRO) and a BD-brand duo-sonic stethoscope.

Height was measured using a professional Sanny stadiometer with 0.1 cm precision. Measurement was

performed with the subjects barefoot and buttocks and shoulders supported in vertical abutment. Total body mass was measured with Filizola digital scales, maximum capacity of 150 kg, measured by INMETRO, with its own certificate specifying a margin of error of ± 100 g.

To measure waist circumference, flexible metallic Starrett tape was used, with measurement definition of 0.1 cm. Abdominal circumference was taken from the lowest curvature located between the ribs and the iliac crest without compressing the tissues. When the slightest curvature could be identified, measurement was taken two centimeters above the umbilical scar. The cut-off points adopted for abdominal circumference were stipulated according to the degree of risk for cardiovascular diseases, namely ≥ 80 cm for women and ≥ 94 cm for men.[12]

BMI was calculated with mass and height measurements, according to the following equation: BMI = mass (kg)/height^2 (cm). The BMI cutoff points adopted were those recommended by the 4th Brazilian Guideline on Dyslipidemia and Prevention of Atherosclerosis, from the Department of Atherosclerosis of the Brazilian Society of Cardiology,[13] that is, low weight (BMI < 18.5); eutrophy (BMI 18.5 – 24.9); overweight (BMI 25 – 29.9) and obesity (BMI ≥ 30).

Five mL of fasting blood sample were collected for the measurement of CRP, total cholesterol and fractions, triglycerides, glycemia and glutamic pyruvic transaminase. The samples were collected by trained professionals in a laboratory environment appropriate for this type of procedure.

PCR was measured by the nephelometry method with plasma serum and precision of 0.1 mg/L. Glycemia, triglycerides, total cholesterol and high-density lipoprotein were obtained by the enzymatic colorimetric method of Trinder. Low-density and very low-density lipoprotein values were obtained by the Friedewald equation. Pyruvic glutamic transaminase was measured by the Reitman-Frankel colorimetric method. Renin was measured by EDTA plasma kinetic radioimmunoassay method.

All volunteers were instructed not to change their diet during the week of collection and to avoid any physical exertion other than usual, as well as not to drink alcohol in the 24 hours prior to the test. NCOCG collection was performed between the fifth and tenth day of the menstrual cycle, considering the lowest hormonal fluctuations, and/or on the 28th day without medication (inactive phase) as recommended by Casazza et al.[14]

Sample size calculation

Sample adequacy calculation was performed with reference to the plasma renin values. A pilot study with six women, three of each group, in which the mean and standard deviation of plasma renin were, respectively, 1.2 ± 0.5 for the NCOCG and 2.6 ± 2.1 for the COCG.

With these data, sample calculation was made in the program GraphPad StatMate 2.0 for Windows, with alpha of 0.05 and beta of 0.8, considering as significant a difference of 0.2 between the groups. The calculation resulted in 21 women in each group. After data collection, a calculation was carried out to determine sample power, which resulted in 0.98.

Statistical analysis

Initially, to determine data distribution, symmetry and kurtosis tests and the Shapiro-Wilk test were conducted. Plasma renin levels did not show normal behavior and were described in median and interquartile range. The other study variables presented normal behavior and were detailed in mean and standard deviation. Abnormal behavior variables were analyzed using the Mann-Whitney test for independent samples. For the variables of usual behavior, unpaired bidirectional Student's t test was used.

Correlation analyses using Spearman's test were conducted between plasma renin and fasting lipid profile variables and plasma renin with PCR. All analyses were performed in the statistical package SPSS (Statistical Package for the Social Sciences) version 13.0, adopting a level of significance of 5%.

Results

Clinical and anthropometric conditions of the sample, 1,970 by 44 women, 22 in each group. Note the homogeneity between the groups, which stands out in the systemic arterial pressure (SBP) (p = 0.02), which is higher in the COCG. A higher level of CRP was also observed in the COCG (< 0.01) (Table 1).

Of the COCs used by the volunteers, 100% contained ethinyl estradiol associated with drospirenone 41% (9), gestodene 27% (6), levonorgestrel 14% (3), chlormadinone acetate 9% (2) and desogrestrel 9% (2). Comparing the fasting lipid variables (Table 2), it can be seen that COCG has a higher triglyceride value (p < 0.01) and total cholesterol (p = 0.02) than NCOCG.

Figure 1 shows the plasma renin value (ng/ml/h) in the groups evaluated. The median and the interquartile deviation of the NCOCG and COCG renin were 0.5 (0.1 – 1.0) and 3.0 (2 – 6), respectively, with significant difference ($p < 0.01$).

Table 1 - Clinical and anthropometric characteristics of the population (n = 44)

Variables	COCG (n = 22)	NCOCG (n = 22)	p value
Age (years)	23 ± 1.3	23 ± 2.0	0.98*
Body mass index (kg/m²)	22 ± 1.4	22 ± 1.0	0.37*
Waist circumference (cm)	73 ± 7.8	70 ± 5.9	0.32*
Systolic blood pressure (mmHg)	119 ± 10.1	107 ± 5.5	0.02*
Diastolic blood pressure (mmHg)	77 ± 6.1	70 ± 10.6	0.18*
C-reactive protein (mg/L)	1.8 (0.5 – 2.2)	0.7 (0.5 – 0.9)	< 0.01#
Glycemia (mg/dL)	82 ± 6.9	83 ± 5.7	0.57*
COC use duration (years)	3.7 ± 2.3		-

*COCG: combined oral contraceptive group; NCOCG: group without combined oral contraceptive. * Two-way t-test for independent samples; # Bidirectional Mann-Whitney test.*

Table 2 - Comparison of fasting lipids (mg/dL) between the groups studied

Variables	COCG (n = 22)	NCOCG (n = 22)	p value
Triglycerides (mg/dL)	88 (72 – 111)	49 (40 – 64)	< 0.01#
Total cholesterol (mg/dL)	207 ± 38.2	183 ± 29.7	0.02*
HDL (mg/dL)	54 ± 13.0	48 ± 11.2	0.10*
LDL (mg/dL)	134 ± 36.4	125 ± 27.2	0.34*

*COCG: combined oral contraceptive group; NCOCG: no combined oral contraceptive group; HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very low-density lipoprotein. * Two-way t-test for independent samples; # Bidirectional Mann-Whitney test.*

There was also a strong positive correlation between CRP and renin ($p < 0.01$ and $r = 0.68$). The correlation between renin and lipid profile variables showed a moderate positive correlation with LDL ($p = 0.01$; $r = 0.46$). There was not any correlation of renin with the other lipid profile variables ($p > 0.05$).

Discussion

Based on the results of this research study, it is possible to suggest that the use of COC may chronically raise plasma renin values in women who use COC. Although the effects of the sociodemographic and nutritional variables of the study population were not evaluated in greater detail (with the quantity, frequency and types of food consumed), sample homogeneity, elimination of confounding factors in volunteer selection and the power obtained after analysis rule out the possibility of type I statistical error, strengthening the study findings.

According to the 7th Hypertension Guideline of the Brazilian Society of Cardiology,[1] the prevalence of systemic arterial hypertension (SAH) among women using COC is 5%. Since the 1990s, studies indicate that the prevalence of SAH is higher in women taking COC than in those who do not use it.[2,3] In our study, we found that SBP is higher in the group that uses COC, although the values are within normal limits.[1]

The reasons why COC use increases BP are not well established. However, abnormalities may be caused by ethinyl estradiol and progestins in the renin-angiotensin-aldosterone system (RAAS).[4]

Synthetic estrogens increase the hepatic synthesis of the renin substrate by inducing the expression of angiotensinogen mRNA.[5] This increase is also accompanied by enhanced renin activity.[6] This, therefore, increases the production of angiotensin II, which in turn is a potent direct and indirect vasoconstrictor, by inducing the production of vasopressin, when binding to the AT1 receptors in the hypothalamus.[7] In addition, angiotensin II, when converted to angiotensin III, induces the production of aldosterone by the adrenals, which in conjunction with increased production of the antidiuretic hormone (vasopressin) enhance the reabsorption of water through the renal tubules. Both vasoconstriction and increased water retention, induced by this system, favor an increase in systemic BP.[7]

On the other hand, progestins, such as drospirenone, present an anti-mineralocorticoid diuretic effect,

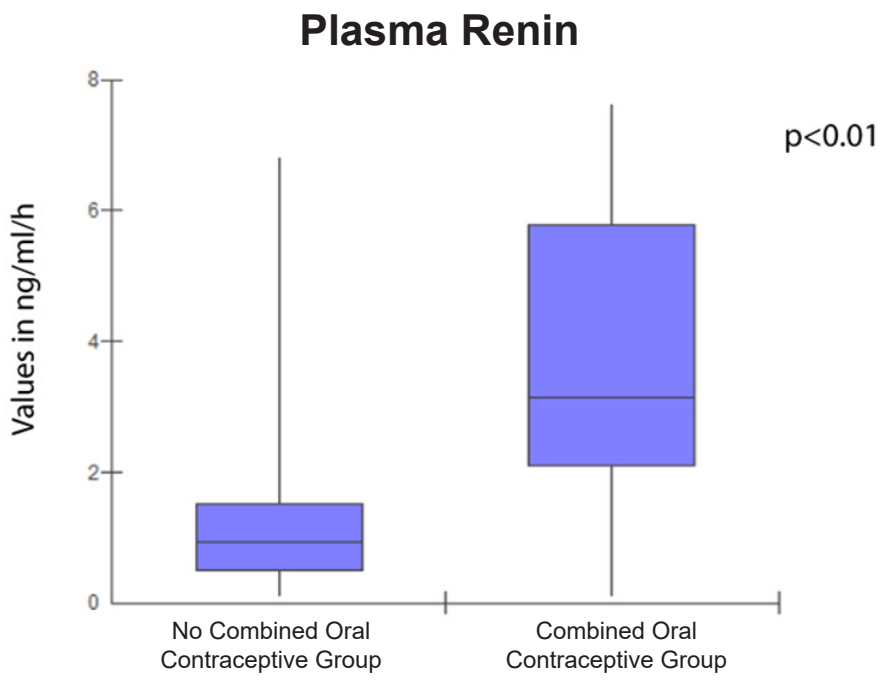

Figure 1 - Median and Interval quartiles of Plasma Renin from the studied groups.

thus reducing BP. In the study by Suthipongse and Taneepanichskul,[8] 120 women were randomized with drospirenone and ethinyl estradiol or levonorgestrel and ethinyl estradiol. The group using drospirenone showed a mean decrease in SBP of 4 mmHg and mean BP lower than the levonorgestrel group. An integrative literature review conducted on articles published between 2012 and 2016, found the same result.[9] In our study, we found that renin values are threefold higher in the COC group. However, because of the sample size, it was not possible to compare the effect of progestin type on plasma renin values.

Increased plasma renin levels increase RAAS activity, which culminates in increased BP. In addition, plasma renin increase is not only associated with increased BP. According to the Framingham study, high plasma renin levels, in addition to increasing RAAS activity, directly contribute to vascular dysfunction, raising the all-cause mortality rate in the general population.[11]

Increased RAAS activity activates other cellular processes, inducing the generation of reactive oxygen species and vascular inflammation that contribute not only to the genesis of hypertension, but also to accelerate damage in the so-called target organs (heart, brain and kidneys).[12]

Studies on inflammation and SAH show a close relationship between infiltration of inflammatory cells and oxidative stress in vascular tissues. RAAS triggers oxidative stress because it stimulates the production of reactive oxygen species such as oxygen superoxide (O2-) and hydrogen peroxide (H2O2). Some studies have contributed to clarifying how RAAS causes elevation in ROS production. Both angiotensin II and aldosterone are capable of inducing the expression of the nicotinamide adenine dinucleotide phosphate oxidase enzyme (NADPH oxidase), a major producer of superoxide anion in vascular tissues.[15]

Nitric oxide, responsible for vasodilation, may have its action altered by the presence of ROS, resulting in increased BP and cellular injury phenomena. This impairment affects the mechanisms of tissue repair and stimulate hyperplasia, hypertrophy and apoptosis, as well as the development of arterial vascular fibrosis.[17] The findings of our study hypothesized that increased renin production in women taking COC promotes increased inflammation and consequently oxidative stress. This

hypothesis is based on the strong positive correlation found between renin and CRP values. We also observed that the CRP values of women using COC were higher than in the non-COC group, corroborating two previous studies produced by our group, which indicates that women using COC have higher subclinical inflammation than women who do not use COC.[11,18]

We can also raise the possibility that high plasma renin levels may re-feed their higher production by stimulating the central nervous system. Increased plasma renin levels by increasing the production of angiotensin II increase sympathetic discharge, since angiotensin II directly stimulates sympathetic activity. Increase in sympathetic activity, in turn, stimulates the beta-adrenergic cells, the glomerular cells of the kidneys, to produce renin.[19]

In summary, increased plasma renin levels appear to be associated with increased subclinical inflammatory activity, which points to the idea that young women with no other risk factors, using COC, are more susceptible to the development of cardiovascular diseases in the medium and long term.

However, in order to assert that COC women are at higher risk of developing cardiovascular diseases, longitudinal studies are necessary to assess cardiovascular dysfunction in this population as primary outcomes. However, it is desirable to evaluate the risks and benefits of prescribing this contraceptive method. Carrying out rigorous clinical follow-up and seeking to evidence potential cardiovascular risk markers, as well as early identifying subclinical inflammation, will be important to prevent the development of cardiovascular diseases in this population in the medium and long term.

Conclusion

Women taking COC have higher serum renin levels and CRP than women who do not use this drug. This points to the possibility that this population is at higher risk of developing systemic arterial hypertension in the long term, which, associated with subclinical inflammation, may increase the risk of cardiovascular diseases.

Limitations

The lack of a detailed nutritional analysis with information on the quantity, type and frequency of carbohydrate consumption, for example, could change some of the evidence, as well as the unchecked sociodemographic aspects.

Author contributions

Conception and design of the research: Oliveira SS, Petto J, Santos ACN. Acquisition of data: Sacramento MS, Santos ACN. Analysis and interpretation of the data: Oliveira SS, Petto J. Statistical analysis: Oliveira SS, Petto J. Análise estatística: Oliveira Writing of the manuscript: Oliveira SS, Petto J, Sacramento MS. Critical revision of the manuscript for intellectual content: Petto J, Ladeia AMT.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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Ethics approval and consent to participate

This study was approved by the Ethics Committee of the *Faculdade Nobre de Feira de Santana* under the protocol number 79549517.3.0000.5654. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

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